ABSTRACT OF THE DISCLOSURE

The invention provides a method of conferring donor CDR binding affinity onto an antibody acceptor variable region framework. The method consists of: (a) constructing a population of altered antibody variable region encoding nucleic acids, said population comprising encoding nucleic acids for an acceptor variable region framework containing a plurality of different amino acids at one or more acceptor framework region amino acid positions and donor CDRs containing a plurality of different amino acids at one or more donor CDR amino acid positions; (b) expressing said population of altered variable region encoding nucleic acids, and (c) identifying one or more altered variable regions having binding affinity substantially the same or greater than the donor CDR variable region. The acceptor variable region framework can be a heavy or light chain variable region framework and the populations of heavy and light chain altered variable regions can be expressed alone to identify heavy or light chains having binding affinity substantially the same or greater than the donor CDR variable region. The populations of heavy and light chains additionally can be coexpressed to identify heteromeric altered variable region binding fragments. The invention also provides a method of simultaneously grafting and optimizing the binding affinity of a variable region binding fragment. The method consists of: (a) constructing a population of altered heavy chain variable region encoding nucleic acids comprising an acceptor variable region framework containing donor CDRs and a plurality of different amino acids at one or more framework region and CDR amino acid positions; (b) constructing a population of altered light chain

variable region encoding nucleic acids comprising an acceptor variable region framework containing donor CDRs and a plurality of different amino acids at one or more framework regions and CDR amino acid positions; (c) coexpressing said populations of heavy and light chain variable region encoding nucleic acids to produce diverse combinations of heteromeric variable region binding fragments, and (d) identifying one or more heteromeric variable region binding fragments having affinity substantially the same or greater than the donor CDR heteromeric variable region binding fragment. A method of optimizing the binding affinity of an antibody variable region is also provided. The method consists of: (a) constructing a population of antibody variable region encoding nucleic acids, said population comprising two or more CDRs containing a plurality of different amino acids at one or more CDR amino acid positions; (b) expressing said population of variable region encoding nucleic acids, and (c) identifying one or more variable regions having binding affinity substantially the same or greater than the donor CDR variable region. The variable region populations can be heavy or light chains and can be expressed as individual populations or they can be coexpressed to produce heteromeric variable region binding fragments.